

*Research Article*

## Effect of size grading on the growth of pirarucu *Arapaima gigas* reared in earthen ponds

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**ABSTRACT.** Size grading is a common management practice in fish farming to minimize growth variability. Such practice has not been established for pirarucu *Arapaima gigas*. Therefore, the present study assessed the effect of size grading on the growth of pirarucu. Juvenile pirarucu were divided into three groups: ungraded fish (U: mean initial weight 1.12 kg), small fish (S: mean initial weight 0.81 kg), and large fish (L: mean initial weight 1.36 kg), and reared for 129 days (phase I) and 158 additional days (phase II), consecutively. No significant difference was observed in the final length, specific growth rate, biomass, survival and feed conversion rate between fish groups. Ungraded fish presented a greater decrease in the coefficient of variation for weight in phase I than in phase II. Size sorting resulted in significantly higher growth after 287 days of farming in the L-graded fish (9.49 kg, final weight), followed by the ungraded group (8.80 kg) and S-graded group (8.01 kg). However, the mean weight of pooled S- and L-graded fish was similar to the ungraded fish group in phase I and phase II, indicating that grading did not improve the general performance of pirarucu in captivity.

**Keywords:** *Arapaima gigas*; growth; production; management; size sorting; size variation

### INTRODUCTION

The aquaculture of pirarucu *Arapaima gigas* (Schinz, 1822) has drawn considerable interest in Brazil and has resulted in increased growth production in the last decade (IBAMA, 2005; IBGE, 2016). Some of the characteristics that encourage the productive sector to invest in the aquaculture of the species are fast growth (up to 10 kg in one year), aerial breathing, and tolerance to high levels of ammonia concentration in the water and white boneless mild-flavor meat (Bard & Imbiriba, 1986; Saint-Paul, 1986; Imbiriba, 2001; Cavero *et al.*, 2004; Fogaça *et al.*, 2011). Considering that pirarucu has been historically subjected to high fishing effort, and now it is classified as a vulnerable species and listed as a threatened species by the Convention on International Trade in Endangered Species of Wild Fauna and Flora; aquaculture is an alternative to provide this fish to the market without threatening natural populations, also reducing pressure on the wild stock.

Intensification of pirarucu farming requires management practices to optimize production. Currently, pirarucu is farmed in earthen ponds, in a two-phase system, as 10 g juveniles are reared up to 1 kg and resorted and reared up to the market size of 10-15 kg (Rebelatto-Junior *et al.*, 2015). Fish reared during long periods and without any management practices to maintain group homogeneity may result in social hierarchy due to differences in weight/length, and sometimes followed by aggressiveness among individuals (Koebele, 1985; Jensen, 1990; Martins *et al.*, 2005). Differences in growth rate are described for several fish species and may be caused by competition for food or other behavioral factors that inhibit growth (Volpato & Fernandes, 1994; Szczepkowski *et al.*, 2011).

Heterogeneous growth and aggressiveness among individuals have been reported for juvenile pirarucu during weaning (Cavero *et al.*, 2003a), and for juveniles (10 g) reared in cages at different stocking densities

(Cavero *et al.*, 2003b). Sorting fish by size (or grading) is a common practice to minimize growth variation along the production cycle of several species (Jensen, 1990; Martins *et al.*, 2005; Onders *et al.*, 2011). One of the positive effects of such practice is better weight gain, as the dominance of large fish over small ones is suppressed when fish are sorted at regular intervals (Baardvik & Jobling, 1990). However, no study is available on the effect of sorting in the production of pirarucu. In this way, the objective of the present study was to assess the effect of size grading of pirarucu reared in earthen ponds upon production efficiency.

## MATERIALS AND METHODS

### Fish stocking and rearing conditions

The study was conducted at Baixão Verde Farm, in the city of Almas, and Serra Azul Farm, in the city of Palmas, both in the state of Tocantins, Brazil, between October 2014 and August 2015. The study complied with the Brazilian guidelines for the care and use of animals for scientific and educational purposes (03/2015). Fish ( $13.4 \pm 2.31$  cm and  $21.22 \pm 9.87$  g) were purchased from a commercial farm and stocked in earthen ponds until they reached mean weight  $\pm$  standard deviation (SD) of  $1.1 \pm 0.3$  kg to the commencement of the experiment. Fish were fed daily a commercial fish feed for carnivorous species (extruded, 40% crude protein) until apparent satiation.

Before the experiment, earthen ponds of  $1,000 \text{ m}^2 \times 1.5$  m depth were wholly drained and sun-dried for seven days and then limed ( $100 \text{ g CaCO}_3 \text{ m}^{-2}$ ) and fertilized ( $3 \text{ g urea m}^{-2}$  and  $10 \text{ g rice bran m}^{-2}$ ) (Lima *et al.*, 2015). Next, ponds were filled with water and fish stocked.

### Experimental design

Fish were divided into three groups: (U) ungraded fish (control), (S) small fish and (L) large fish, in duplicate (one replicate per farm), totaling six experimental units (Wallat *et al.*, 2005) stocked at density of 1,000 fish  $\text{ha}^{-1}$  (100 fishes per pond), suitable for commercial production (Ono & Kedhi, 2013). The study was divided into phase I (129 days), when fish were randomly assigned to the ungraded group ( $1.12 \pm 0.05$  kg), and sorted by hand to the S- ( $0.81 \pm 0.01$  kg) and L- ( $1.36 \pm 0.33$  kg) graded groups with weight below and above the mean weight of the unsorted group, respectively (Wallace & Kolbeinshavn, 1988); and phase II (158 days), when only S- and L-graded fish groups were graded again. In this way, fish were reared for a total of 287 days, which added to 90 days in the nursery phase, reached the recommendation of one year farming in captivity (Imbiriba, 2001).

### Feeding

During the experiment, fish were fed twice daily (07:00 and 17:00 h), a commercial extruded feed for carnivorous species (40% crude protein). Feed portions were thoroughly distributed on the water surface in each pond. In the first 45 days, fish were fed at a rate of 3% biomass  $\text{d}^{-1}$ , then 2.5% biomass  $\text{d}^{-1}$  until the end of phase I (129 days total). During phase II, fish were fed at a rate of 1.8% biomass  $\text{d}^{-1}$ . All fish were individually measured and weighed at the beginning and end of the experiment. Intermediate measurements were taken at days 40, 80, 129, 177 and 234, when 30 fish per pond were randomly captured with a dragnet, and individually measured and weighed.

### Hierarchy assessment

Twenty fish per experimental unit was marked by PIT (passive integrated transponder) tags at the beginning of the experiment to assess the hierarchical fish position during the study period. For each morphometric measurement, fish were classified as subordinate or dominant when lower or higher in weight, respectively, than the mean weight of the group (Cutts *et al.*, 1998; Martins *et al.*, 2005). During the study period, fish were rated as remaining in the position (same), became dominant (up), became subordinate (down) and varied between the dominant and the subordinate groups (varied).

### Growth and water quality parameters

Fish growth performance was assessed based on mean final weight, final biomass, apparent feed conversion ratio ( $\text{FCR} = \text{total feed intake}/\text{total weight gain}$ ), coefficient of variation for weight and length ( $\text{CV} = \text{standard deviation}/\text{mean weight or length} \times 100$ ), specific growth rate ( $\text{SGR} = 100 \times (\ln \text{final weight} - \ln \text{initial weight})/\text{days}$ ) and survival ( $100 \times (\text{final number of fish}/\text{initial number of fish})$ ). For each experimental unit, weight and length data were regressed ( $W = aL^b$ , where  $W$  is weight,  $L$  is the length,  $a$  is the constant, and  $b$  is the regression exponent). The regression exponent values were compared between experimental units for significant differences (Le Cren, 1951). The fish condition factor ( $K$ ) was calculated based on the exponential regression index ( $K = W/aL^b$ ) (Le Cren, 1951). To assess water quality parameters, temperature and transparency were measured every two days with a thermometer and a Secchi disc, respectively; water pH, ammonia and alkalinity were measured monthly with a commercial colorimetric kit (Alfakit<sup>®</sup>, Florianópolis, SC, Brazil).

### Statistical analysis

For statistical analysis of each variable, the means of each pond was considered the experimental unit, and

each propriety was considered a block. Data were submitted to analysis of variance and Tukey test ( $P < 0.05$ ). When premises of normality (Shapiro-Wilk) and homogeneity (Bartlett) were not met, data were transformed (Box & Cox, 1964) or compared by the non-parametric test of Kruskal-Wallis. Values are expressed as mean  $\pm$  standard deviation. Statistical analysis was performed in R (R Core Team, 2016).

## RESULTS

### Fish growth performance

Growth performance (weight, length, biomass, SGR, survival and FCR) of pirarucu *Arapaima gigas* reared in earthen ponds and submitted to different size grading practices are presented in Table 1. The mean pooled weight of small and large fish (1.09 kg) at the beginning of the experiment did not differ ( $P = 0.384$ ) from the ungraded fish mean weight (1.12 kg), considering that fish graded as small or large derived from the ungraded fish group. The mean pooled weight of small and large-graded fish (4.38 kg) did not differ ( $P = 0.223$ ) from the ungraded group (4.35 kg) by the end of either phase I or phase II (8.75 vs. 8.80 kg, respectively) ( $P = 0.476$ ). Fish size grading only resulted in better performance after 287 days of culture with higher final weight for L-graded fish, followed by ungraded fish, whereas S-graded fish presented the lowest final weight. Despite the significant difference in the final weight of fish by the end of phase II, the final weight of the ungraded group did not differ from the pooled mean final weight of the S- and L-graded groups, which indicates that this practice did not improve pirarucu growth performance in captivity.

In phase I, fish SGR did not differ between groups, and it was 21% higher in the S-graded fish than the other groups. In phase II, no significant differences were observed, but the SGR was higher in L-graded fish and lowered in the S-graded fish group. From phase I to phase II, the SGR was significantly reduced only for the S-graded group ( $P = 0.0389$ ). FCR and survival were similar in all groups.

### Growth curve and condition factor

The confidence interval of the fish growth curves in the different treatments overlapped during most of the study period, only separating towards the end (Fig. 1a) with a significant difference in the final fish weight.

Fish weight and length were adjusted in a curve for each treatment, and the exponential index (a and b) of the equations were compared (Table 1). Since no significant difference was observed, a single exponential curve was adjusted with all data on weight and

length, resulting in the equation  $Y = 0.0027X^{3.2656}$ , where Y is weight and X is the length ( $P < 0.05$ ), and  $R^2 = 98.2\%$  (Fig. 1b). The curve exponent indicated allometric growth for pirarucu, *i.e.*, higher increment in weight than in length when reared in earthen pond. The condition factors for the fish in the three groups, calculated from the regression indexes, were not significantly different (Fig. 2).

### Heterogeneous growth

The coefficient of variation (CV) used to evaluate growth heterogeneity in each group was high at the beginning of the experiment and tended to decrease in all treatments in both culture phases, but with sharper descent in phase I (Fig. 3). No significant difference was observed for the CV between groups ( $P = 0.446$ ) or between the initial and final values in phase I or phase II ( $P > 0.05$ ), except for the lower CV of the L-graded fish weight at the end of phase II ( $P = 0.048$ ). In general, ungraded fish presented a sharper reduction in the CV of weight in phase I, approaching values of S and L-graded fish, demonstrating the natural trend in pirarucu to reduce CV. As for length, in phase I, CV for ungraded fish did not vary, whereas it was reduced for S- and L-graded fish. In phase II, a less pronounced and similar reduction in weight and length CV between groups was observed. A higher weight variability trend was observed in the initial phases of pirarucu farming.

### Hierarchy

In the ungraded fish group, more individuals remained in the same hierarchical position as at the beginning of the experiment, demonstrating low variation in the social rank established. On the other hand, among the L-graded fish, 50% remained in the same position, whereas 16.7% increased their social status. Among the S-graded fish group, 27.2% increased their social status, whereas 20.8% varied their hierarchical position more than once during the study. A similar proportion of fish decreased their hierarchical position in all the treatments (Fig. 4).

### Water quality

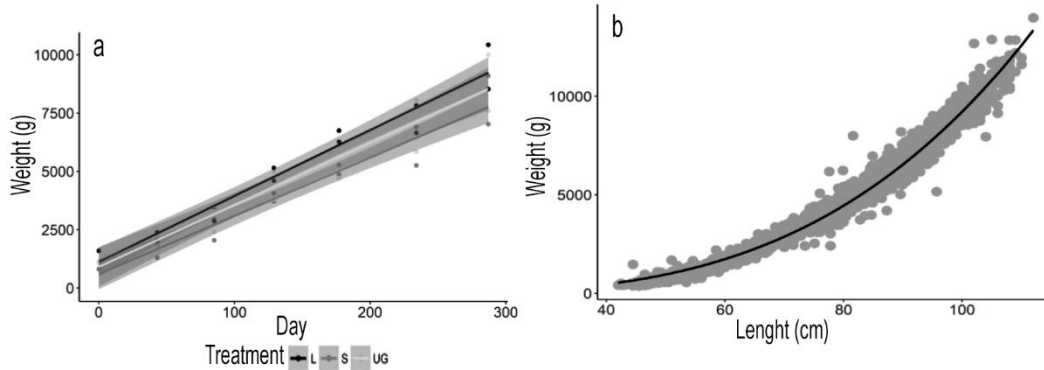
No significant differences were observed for any of the water quality parameters (Table 2), demonstrating that size grading, and even grouping fish of different mean weight in different ponds did not affect water quality.

## DISCUSSION

Water quality parameters were within recommended values for tropical fish species (Boyd & Lichtkoppler, 1979) and considered adequate for the farming of pirarucu *Arapaima gigas* in captivity (Ono & Kedhi, 2013). As previously reported for yellow perch *Perca*

**Table 1.** Growth performance (initial and final weight and length, biomass, specific growth rate (SGR), survival and feed conversion ratio) of pirarucu *Arapaima gigas* reared in earthen ponds for 287 days and size graded. Mean values followed by different letters in the same row are significantly different (Tukey test,  $P < 0.05$ ). SGR is the specific growth rate; b is the exponent of the exponential regression, analyzed by Kruskal Wallis. \*The value of each variable is a mean of all fish in the experimental units of each treatment.

Parameter	Size grading			P-value (Treatment)	P-value (Block)
	Ungraded	S-graded	L-graded		
Phase I - 0 to 129 days					
Initial weight* (kg)	1.12 ± 0.05	0.81 ± 0.01	1.36 ± 0.33	0.16146	0.32635
Final weight* (kg)	4.35 ± 0.85	3.88 ± 0.25	4.87 ± 0.38	0.43606	0.57284
Initial length* (cm)	54.53 ± 0.52	50.86 ± 0.62	57.64 ± 3.05	0.08215	0.23386
Final length* (cm)	9.85 ± 4.70	76.00 ± 3.00	81.35 ± 0.80	0.34242	0.29130
SGR (%)	1.05 ± 0.18	1.215 ± 0.06	1.00 ± 0.13	0.10040	0.05837
Phase II - 130 to 287 days					
Initial weight* (kg)	4.35 ± 0.85	3.76 ± 0.19	4.99 ± 0.29	0.3038	0.52793
Final weight* (kg)	8.80 ± 1.69ab	8.01 ± 1.46 b	9.49 ± 1.34 a	0.03099	0.004778
Initial length* (cm)	79.90 ± 4.73	75.15 ± 2.56	82.12 ± 0.17	0.1902	0.2368
Final length* (cm)	97.30 ± 7.156	95.20 ± 8.22	99.79 ± 5.81	0.120861	0.009565
SGR (%)	0.660 ± 0.001	0.605 ± 0.12	0.720 ± 0.19	0.58817	0.19936
General - 0 to 287 days					
Final biomass (kg)	878.41 ± 193.66	727.52 ± 382.43	847.65 ± 187.82	0.49083	0.05762
Survival (%)	78.00 ± 19.80	72.40 ± 7.40	76.40 ± 1.70	0.8750	0.3064
Feed Conversion	2.75 ± 1.09	2.82 ± 0.64	2.85 ± 0.35	0.9665	0.0855
b	3.46	3.60	3.59	0.1712	-

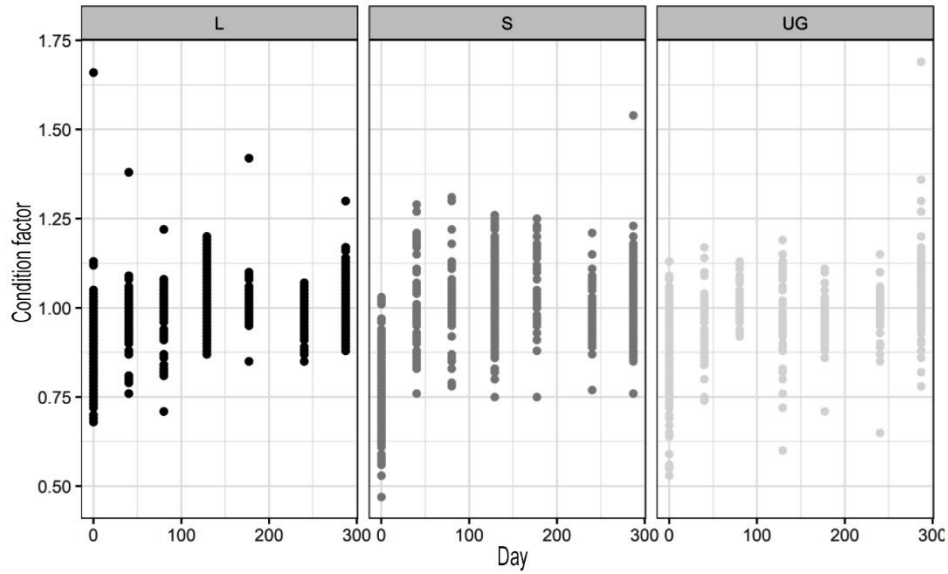


**Figure 1.** a) Weight gain, b) length-weight relationship of pirarucu *Arapaima gigas* reared in earthen ponds for 287 days and subjected to size grading.

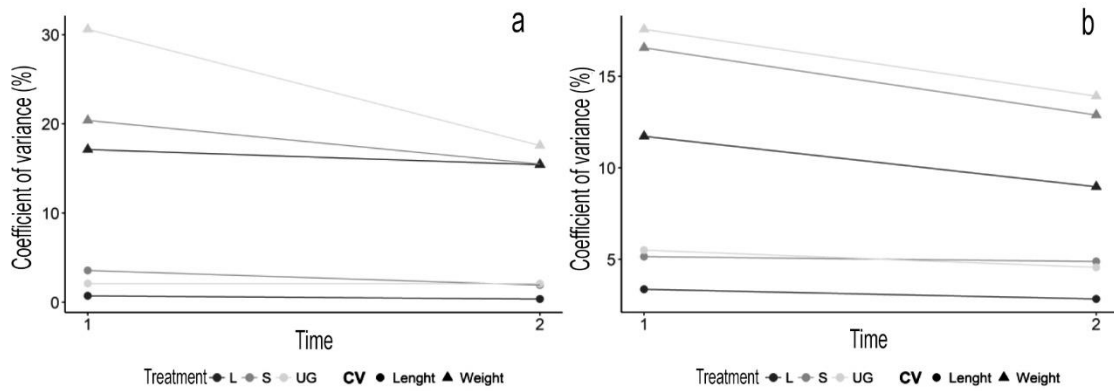
*flavescens* (Wallat *et al.*, 2005), despite resulting in different biomasses, size grading for pirarucu did not influence water quality parameters.

Although not statistically significant, there was a trend which suggested that L-graded fish presented higher final weight by the end of phase II than the S-graded or ungraded fish groups, similar result reported for the North African catfish *Clarias gariepinus* (Burchell, 1822) (Martins *et al.*, 2005) and paddlefish *Polyodon spathula* (Walbaum, 1792) (Onders *et al.*, 2011) subjected to size grading. Considering that size

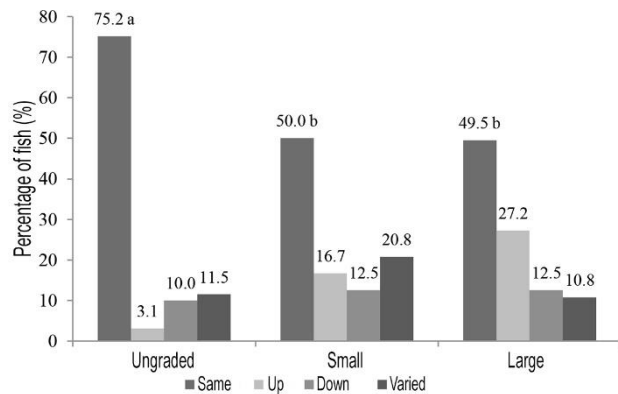
grading aims at reducing social dominances and allow fish to better express their potential growth (Jobling & Reinsnes, 1987; Johnson, 1997; Martins *et al.*, 2005), it was expected that by the end of the culture phases, compensatory growth would be observed in the graded fish. Nevertheless, pooled S- and L-graded fish weight did not differ from the ungraded fish, which demonstrates that size grading did not favor general growth performance of pirarucu, the same as observed for *Xiphophorus helleri* Heckel, 1848 (Endemann *et al.*, 1997), North African catfish (Martins *et al.*, 2005) and



**Figure 2.** Condition factor of pirarucu *Arapaima gigas* reared in earthen ponds for 287 days and subjected to size grading. Small (S), large (L), and ungraded (UG) fish groups.



**Figure 3.** Heterogeneous weight gain and length of pirarucu *Arapaima gigas* reared in earthen ponds and subjected to size grading in two production phases: a) until 129 days, and b) from 130 to 287 days. The coefficient of variation (CV) is presented for weight and length. L, S, and UG represent fish groups graded as large, small and ungraded, respectively.



**Figure 4.** Change in hierarchical position (%) of pirarucu *Arapaima gigas* reared in earthen ponds for 287 days and subjected to size grading. Means followed by different letters are significantly different (Tukey test,  $P < 0.05$ ).

Arctic charr *Salvelinus alpinus* (Linnaeus, 1758) (Wallace & Kolbeinshavn, 1988). On the other hand, no losses in productive performance were observed, as reported for *S. alpinus* (Baardvik & Jobling, 1990) and Atlantic halibut *Hippoglossus hippoglossus* (Linnaeus, 1758) (Stefansson *et al.*, 2000). In this way, size grading during the pirarucu grow-out phase in earthen ponds did not improve production efficiency (Zakes *et al.*, 2004), and it is not a practice indicated for the species and the production phases as assessed in conditions similar to commercial farming. Wallace & Kolbeinshavn (1988) have remarked that most of the studies concluding that grading is a feasible management practice were done in a laboratory-scale with few animals.

**Table 2.** Water quality parameters for pirarucu *Arapaima gigas* reared in earthen ponds for 287 days and subjected to size grading.

Parameter	Size grading			P-value (Treatment)	P-value (Block)
	Ungraded	S-graded	L-graded		
Temperature (°C)	26.42 ± 2.78	26.33 ± 2.98	26.37 ± 2.91	0.7367	0.0005
Transparency (cm)	65.58 ± 35.52	71.64 ± 22.64	69.08 ± 44.86	0.8705	0.0334
Ammonia (mg NH <sub>4</sub> L <sup>-1</sup> )	0.15 ± 0.09	0.17 ± 0.05	0.09 ± 0.02	0.4858	0.3701
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	18.57 ± 10.71	25.26 ± 16.81	24.38 ± 15.73	0.2863	0.0166
pH	7.18 ± 0.78	6.98 ± 0.85	7.38 ± 1.59	0.7223	0.0541
Carbon dioxide (mg CO <sub>2</sub> L <sup>-1</sup> )	3.51 ± 1.86	4.32 ± 1.11	2.15 ± 3.04	0.2815	0.0702
O <sub>2</sub> (mg O <sub>2</sub> L <sup>-1</sup> )	7.12 ± 1.96	6.45 ± 1.33	7.83 ± 2.68	0.3295	0.0367

Although not statistically significant, the trend of higher growth rate and a high percentage of individuals increasing hierarchical position in the S-graded fish group suggest compensatory growth in the first rearing phase, favored by the absence of large fish (Barki *et al.*, 2000). This behavior was not observed in the second phase, when L-graded fish presented a trend of a higher growth rate, indicating that the presence of large fish only suppressed growth in the initial phases. Nevertheless, Sunde *et al.* (1998) only observed a higher growth rate in S-graded *Scophthalmus maximus* (Linnaeus, 1758) towards the end of the study period, which was explained as size-specific growth. Reduction in SGR as they grow is a common behavior in fish (Jobling, 1985; Sunde *et al.*, 1998; Sun & Chen, 2014) and observed in the present study in all treatments, with significance only in the S-graded group. Pirarucu survival and feed conversion were not influenced by grading, corroborating to results reported for yellow perch (Wallat *et al.*, 2005), turbot *Scophthalmus maximus* (Sunde *et al.*, 1998) and North African catfish (Martins *et al.*, 2005).

The weight-length relationship in pirarucu presented an exponential coefficient of 3.2656, which indicates a positive allometric growth for the species in the rearing conditions of the present study. Considering that the exponent may vary according to some factors, such as season, population and environmental conditions (Froese, 2006), pirarucu reared in earthen ponds present increased weight gain than length during the production cycle, as reported as a common characteristic for several fish species (Cifuentes *et al.*, 2012).

High CV was observed in pirarucu weight at the beginning of the study, mainly in the ungraded fish group, corroborating data presented by Cavero *et al.* (2003b) for small pirarucu (approximately 100 g) and by Gjerdrem (2005), who reported CV values for fish between 20 and 35%, which are higher than values for most of the farmed animals (7 to 10%). It is expected that heterogeneous fish size groups result in high

variability by the end of the culture period (Smith & Fuiman, 2003). However, for pirarucu, the CV values by the end of the production phases were similar between graded and ungraded fish, which has been reported for paddlefish reared in earthen ponds (Onders *et al.*, 2011). According to Volpato & Fernandes (1994), the increase in the CV may occur following size grading procedure, but it was not observed for pirarucu in the phases tested in the present study. Along the culture period, a trend of decrease in CV was observed, as reported for silver perch *Bidyanus bidyanus* (Barki *et al.*, 2000) and yellow perch reared for 12 months (Wallat *et al.*, 2005). Accordingly, the trend of pirarucu to reduce growth CV along the culture cycle was observed, typical behavior of gregarious fish species (Yamagashi, 1969), which supports the fact that grading pirarucu does not improve productivity.

As regards hierarchical position, within size graded fish, more individuals increased social status, as described by Martins *et al.* (2005) for the North African catfish. After grading, there might be more social interaction between individuals of the same size and stimulate new social hierarchical arrangements (Volpato & Fernandes, 1994; Zakes *et al.*, 2004). On the other hand, a highly heterogeneous fish (ungraded) group led the individuals to remain in the same hierarchical position, as the small fish are inhibited by the presence of larger conspecifics (Mgaya & Mercer, 1995).

The growth potential in captivity is one of the main characteristics that attract investments from the productive sector to the production of pirarucu (FAO, 2012). Such potential has been observed in the present study, as fish final weight was of 8.0 to 9.5 kg in 287 days of culture, which are very high values compared to the main fish species farmed in Brazil, such as Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) and tambaqui *Colossoma macropomum* (Cuvier, 1816) (Lima *et al.*, 2013). Additionally, the market value of pirarucu (US\$ 2.81 kg<sup>-1</sup>) is also higher than tilapia (US\$ 1.49 kg<sup>-1</sup>) and tambaqui (US\$ 1.72 kg<sup>-1</sup>) (IBGE, 2016).

Pirarucu growth performance was even higher than reported by Pereira-Filho *et al.* (2003), who reared 133 g pirarucu in an earthen pond for 12 months to a final weight of 7.0 kg. In this case, the stocking density that was three times higher in the study by Pereira-Filho *et al.* (2003) may have caused such difference.

Finally, the size grading of pirarucu did not improve growth performance, and it may be used to form groups of fish to reach the market size faster, as suggested by Wallat *et al.* (2005). However, it should be taken into account that, further to not improving fish growth, grading may increase production costs, increase diseases due to handling, reduce growth, and even loss of individuals (Sunde *et al.*, 1998).

### CONCLUSION

Size grading in pirarucu *Arapaima gigas* resulted in higher final weight in the L-graded fish group, followed by the ungraded and S-graded fish groups after 287 days of culture. Nevertheless, the pooled final weight of S- and L-graded fish did not differ from the ungraded fish group, which shows that size grading does not improve general performance in pirarucu. However, it may be used to form groups of fish to reach the commercial size faster or to produce a more uniform size fish if that is desired by the wholesalers/processors/market.

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